Material and Methods:

Materials and Methods are available in the online- only Data Supplement

Study design: Patients included in this study were selected from the following studies at the University of Pennsylvania: University of Pennsylvania (UPenn) High HDL Cholesterol Study (HHDL), UPenn Catheterization cohort (PennCATH), High HDL and CHD study (HCAD), and Philadelphia Area Metabolic Syndrome Network (PAMSyN). HHDL is a cross-sectional study of genetic factors contributing to elevated HDL-C levels. Individuals with HDL-C levels ≥ 90th percentile for age, race, and gender were identified by physician referrals or through the Hospital of the UPenn clinical laboratory. PennCATH is a study of subjects undergoing coronary angiography at UPenn Health System hospitals and has been previously described[1]. HCAD is a cross-sectional, observational study of subjects with HDL-C > 90th percentile and coronary heart disease. PAMSyN is a cross-sectional study of individuals with varying numbers of metabolic syndrome criteria, from none to all 5.

We identified patients that previously participated in clinical studies at the University of Pennsylvania and selected individuals with HDL-C levels above the 90th percentile with documented cardiovascular disease defined as either a history of heart attack, angioplasty, coronary artery bypass surgery, coronary calcium score above the 90th percentile, or greater than 50% stenosis on CT angiogram. We excluded subjects with plasma LDL-C level greater than 190 mg/dL, triglycerides greater than 400 mg/dL, diabetes (type I and type II), history of liver disease with LFTs greater than twice the upper limit of normal, history of kidney disease or chronic renal insufficiency, and use of medications known to significantly affect HDL-C levels, specifically including niacin doses greater than 1500 mg daily[2]. Controls were selected based on the absence of CAD and matched to cases for race, gender, and HDL-C level within 10 mg/dL. Controls were selected to be the same age or up to 10 years older than the cases. Additional exclusion criteria for controls included history of stroke, transient ischemic attack, and history of abdominal aortic aneurysm. Cases were included retrospectively and data (i.e. medications, age of onset of coronary disease) was self-reported via a survey.

Lipid and apolipoprotein measurements: Plasma concentrations of total cholesterol, HDL-C, triglycerides, and apolipoproteins were measured using blood samples obtained after a minimum of an 8-hour fast using CDC-standardized methods. Measurements were performed on frozen (−80°C) EDTA plasma and serum. The Friedewald equation was used to determine the amount of LDL-C. HDL phospholipid (HDL- PL) was determined by phosphotungstanate precipitation of LDL and VLDL and subsequent measurement of the phospholipid component in the HDL particles in the remaining supernatant. Non HDL- PL was calculated by subtracting the HDL- PL from the total phospholipid content.

Radiolabeled cholesterol efflux: Cholesterol efflux capacity was measured in patient samples. J774 mouse macrophage cells were plated and labeled with 2μCi of ^{3}H cholesterol per milliliter overnight. Cells were then incubated for 6 hours in either the

presence or absence of 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP, an upregulator of ATP-binding cassette transporter-1 (ABCA1)[3]. ApoB containing proteins were removed from plasma by polyethylene glycol precipitation. Efflux media containing the equivalent of 1% apolipoprotein B–depleted serum or plasma was then incubated for 2 hours at 37°C. Each patient sample was run in duplicate in both the presence and the absence of cyclic AMP (cAMP). Media was collected and radioactivity determined by liquid scintillation counting after passing through a 0.22 μM filter. Efflux to media without serum was used as a baseline control. The quantity of radioactive cholesterol incorporated into cellular lipids was determined after isopropanol extraction[4]. Percent efflux was calculated by the formula: [(cpm of ^{3}H cholesterol in the media - cpm of ^{3}H cholesterol in serum free media) / (cpm of ^{3}H cholesterol in the cells + cpm of ^{3}H cholesterol in the media)] × 100. A pooled plasma control was included on each plate to which samples from patients were normalized. - mediated cholesterol efflux capacity was determined by subtracting the basal cholesterol efflux capacity (without cAMP) from the total cholesterol efflux capacity (with cAMP).

Cholesterol esterification rate: Patient plasma or serum samples were equilibrated overnight at 4°C in the presence of ^{3}H cholesterol in duplicates. Samples were then incubated at 37°C for two hours. Ethanol was added to each sample to terminate the esterification reaction. Free cholesterol and cholesterol ester fractions were subsequently separated by column chromatography. Liquid scintillation counting was used to determine radioactivity in both the free cholesterol and the cholesterol ester fraction of the sample. Cholesterol esterification rate (nmol/hr/ml) = (Free cholesterol concentration) x (% free cholesterol esterified)[5]. Each sample was run in duplicates, and a control plasma sample was included in each assay, to which all of the patient samples were normalized to control for inter-assay variation,

Phospholipid transfer protein (PLTP) activity: PLTP activity was measured using the Kamiya Biomedical PLTP activity assay (Cat. No. KT-206).

Nuclear Magnetic Resonance (NMR) spectroscopy: Particle size and GlycA were measured by NMR spectroscopy using the LipoProfile-3 algorithm at LipoScience, Inc. (Raleigh, NC). VLDL, LDL, IDL, and HDL subclasses of different size were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Large HDL particle subclass diameters range from 9.4 nm to 14 nm, medium HDL particle subclass diameters range from 8.2 nm to 9.4 nm, and small HDL particle subclass diameters range from 7.3 nm to 8.2 nm.

Statistical Analysis:

Data was examined using descriptive statistics. Continuous variables were summarized by the min, max, mean, median, and standard error. Categorical variables were summarized by frequency and percentage. Data was modeled using a logistic regression with the case/control as binary response variables. The R software was used for the analysis and the p value was evaluated for each predictor variable. The analysis was performed without any covariate adjustment.

References:

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